Study The Role of Polyphenolic Extract of Capparis Spinosa L. Leaves As a Hypoglycemic agent

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A B S T R A C T

The present study investigate the effect of polyphenolic extracts of Capparis Spinosa L. leaves on some biochemical parameters in female rats in vitro. The work involved the following studies: Analytical study: involved extraction, isolation and identification of polyphenolic content of Capparis Spinosa L. extract. The UV-Vis Spectra and high performance liquid chromatography of extracted polyphenols are proved the presence of (Gallic acid, Caffeic acid, Coumaric acid, Vanillic acid, Syringic acid, Ferulic acid, Chlorogenic acid, Rutin and Quercetin) in the extract. Biochemical parameters: all rats in bio-chemical parameters are divided into 4 groups, group (A) positive control (normal) that were treated with distill water (D.W), negative control group(B) that were treated with alloxan (125mg/kg) B.W. only diabetes rats that were treated with (15 mg/kg) B.W. of Capparis spinosa L. extract for 4 weeks, and group (D) alloxan-induced diabetes rats that were treated with (25 mg/kg) B.W. of Capparis spinosa L. extract for (4 weeks). The results indicated that group (B) showed a significant increase (P <0.01) in serum glucose compared with control group (A). The rats were fed with plant extract groups(C and D) show a significant decrease (P<0.01) in the level of serum glucose in comparison to diabetic animal group(B).

Keywords: Capparis Spinosa L., polyphenols, Leaves, hypoglycemia .

Introduction:

Diabetes mellitus is a disease commone in all partes of the world (Anonymous, 2007). It is defined as a group of disorders characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins (Patel et al., 2011; Warjeet, 2011). Chronic hyperglycemia is considered a major initiator of diabetic complications (Rama et al., 2009) ,and causes damage to eyes, kidneys, nerves, heart and blood vessels (Mayfield, 1998). It is becoming the third “killer” of the health of mankind along with cancer cardiovascular and cerebrovascular diseases (Chauhan et al., 2010). Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin (Maiti et al., 2004). The increasing proportion of the aging population, consumption of calorie rich diet, obesity and sedentary lifestyle have led to a tremendous increase in the number of diabetics worldwide (Vats et al., 2004). The word “diabetes” is derived from the Greek word “Diab” (meaning to pass through, referring to the cycle of heavy thirst and frequent urination); “mellitus is the Latin word for “sweetened with honey” (refers to the presence of sugar in the urine) (Warjeet Singh, 2011). Diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defenses (Memisogullari and Bakan, 2004).
The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine (Wadkar et al., 2008). The World Health Organisation not only encourages the use of plant medicines, but also recommended scientific evaluation of the hypoglycaemic properties of plant extracts (Dirks, 2004).

Phenolic compounds are found in almost every plant-derived food. Fruits and vegetables contain several thousand structurally diverse phytochemicals, of which a large fraction are polyphenols (Scalbert and Williamson, 2000). The beneficial effects of polyphenols are mainly attributed to their antioxidant properties, many dietary polyphenols are known antioxidants (Surh, 2003; Pan and Ho, 2008; Miller and Ruiz-Larrea, 2002). Polyphenols exhibit multiple pharmacological properties such as antimicrobial, anti-allergic, anti-ulcerogenic, anti-neo plastic, and anti-inflammatory activities (Formica and Regelson, 1995). The effects of polyphenols therapeutically relevant for the biological systems, are: they reduce the scavenger properties for oxygen free radicals (Burns, 2000), they reduce platelet aggregability (Jeong et al., 1999), and they decrease arterial blood pressure (Hiroko et al., 2004). The bioavailability and biological properties of dietary polyphenols vary to a great extent and depend on their chemical structure (Manach et al., 2005; Loke et al., 2008).

**Capparis Spinosa L. plant**

*Capparis spinosa L.* is well known with its common name ‘Capers’ in different countries (Azaizeh et al., 2003). This plant also known as the caper bush, is a perennial winter deciduous species that bears rounded, fleshy leaves and large white to pinkish flowers (Ramezani et al., 2008). Extracts of different parts of *C. spinosa* have been shown to possess biological activity against a large number of pathogens (Chopra et al., 1996). Antifungal, antibacterial, anti-amoebic, and anti-worm activities have been demonstrated (Asolkar et al., 1992; Guba Bakshi et al., 1999), antidiabetic, antihyperlipidemic (Eddouks et al., 2005), anti hypertensive, poultice (Baytop et al., 1984) antileishmania, antihepatotoxic, , and antiallergic activities (Jacobson and Schlein, 1999; Gadgoli et al., 1999; Trombetta et al., 2005).

**Materials and Methods:**

**Study Plant**

*Capparis spinosa L.* leaves were collected in May- 2013 from Nasiriyah city at Iraq, then it was authenticated and specimen of plant was classified in biological department-college of science at university of Thi qar in Iraq by Asst. prof. Hayder Radhi. The leaves were cleaned, washed by distilled water, dried at room temperature for two weeks, ground as powder and kept in Dark glass containers for further use.
Chemicals

Chloroform, ethanol, sodium hydroxide, hydrochloric acid, ferric chloride, acetic acid, lead acetate, - naphthol, sulphuric acid, Potassium citrate, mercuric chloride, Potassium hydroxide, n-hexane.

Extraction of Polyphenols from *Capparis spinosa* L Leaves

(500 g) of the powder dry leaves were defatted by washing several times with hexane(1L) at (60°C) , then it was macerated with (800mL) of acetic acid (2% v/v), the mixture were placed in sterile conical flask volume (2000mL) and put in water bath(60°C) for 8h, then the extraction process done by reflex condenser. The mixture was heated at50 C and then left to cool. The suspension was filtered by Buchner funnel by Whatman no.1 filter paper and use vacuum pump. The precipitate was canceled and the filtrate volume was measured then n-propanol was added into filtrate with the same volume of filtrate. Then (NaCl) added until to become solution super saturated. Then, it was evaporator by using rotary evaporator until drying (Gayon, 1972).

Primary Qualitative Analysis

Isolated polyphenols were underwent a number of different tests such as:

**Phenolic compounds test:** was carry out by using (1%) ferric chloride. (Waterman and Mole, 1994).

**Flavonoids test:** was achieved by using(5N) alcoholic potassium hydroxide ( Al- Assadi, 2001)

**Tannins:** was achieved by using (1%) lead acetate (Molan et al., 1997).

**Carbohydrates test:** was done by using Molish’s reagent (Harborne, 1984).

**Glycosides test:** was carried out by using Benedict’S reagent (Harborne, 1984).

**Alkaloids test:** was done by using Wagner’S reagent (Harborne, 1984).

**Saponins test:** was carried out by using(5%) mercuric chloride (Harborne, 1984).

**Triterpinoids test:** was achieved by using concentration sulfuric acid(Harborne, 1984).

**Triterpines and Setrols test:** was achieved by using Liebermann- Burchard reagent. (Harborne, 1984).

Investigation of Polyphenolic Extract by UV-VIS (spectrophotometer)

The absorption spectra of plant constituents was measured in very dilute solution against a blank solvent by using an automatic recording spectrophotometer. The solvent was used for UV spectroscopy is water : the method was performed by using polyphenolic. The sample solutions absorbance (A), was recorded by measuring the range scan from 190nm to800nm on a double beam UV-VIS spectrophotometer (Ikbal, 2004).

Investigation of Polyphenolic Extract by HPLC Technique

The extract were separated on FLC ( Fast Liquid Chromatography) column, C-18, 3 µm particle size (50×4.6 mm ID), mobile phase were 0.1% acetic acid in Dionized water: acetonitrile (20:80V/V) using linear gradients from 0-100%B in 10 minutes, detection UV set at 275 nm, flow rate 1.5 mL/min, the sequences of the eluted material of the standard were as follow, each standard was 25 µg/mL. 1.0g of the sample was weighted, then
dissolved in 10 mL HPLC methanol, the sample shaking and agitated in ultrasonic bath for 10 minutes, then concentrated by evaporating the solvent with stream of liquid N₂ until reach 0.2 µm (supelco company cat No16534K) then 20 µL were injected on HPLC column. The concentration for each compound were quantitatively determined by comparison the peak area of the standard with that of the samples. The concentration for each compound were quantitatively determined by comparison the peak area of the standard with that of the samples. The separation occurred on liquid chromatography Shimadzu 10 AV-LC equipped with binary delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by UV-Vis 10 A-SPD spectrophotometer (Ikbal, 2004).

Animals and Housing

Twenty healthy adult female rats weighing (100-169 g) of 9-10 weeks old were used in the present study. Animals were housed in the animal house of biology Dept. College of Education, Thi-Qar University. Experiments were achieved between September-2013 & October-2013. Animals were housed in iron boxes bedded with wooden chips. During the experimental period six animals were kept in each box and they were housed under standard laboratory conditions (12h light: 12h dark photoperiod (LD) at 22 ± 2 C° and relative humidity 45-55% (Coskun et al, 2004). Animals were fed on standard rat pellet and tap water ad libitum. The standard pellet contains wheat 66.6%, soya 25.6%, and sunflower oil 4.4%, lime stone 1.5%, salt 0.63%, methionine 0.158%, choline chloride 0.062% and trace elements 0.05% (Krinke, 2000).

Administration of Laboratory Animals:

Experimental animals were divided into four groups (5 rats in each group) upon the following designed:

- **Group A**: positive control (normal) that were treated with distill water (D.W).
- **Group B**: negative control that were treated with alloxan (125mg/kg) B.W. only to induction of diabetes.
- **Group C**: alloxan-induced diabetes rats that were treated with (15 mg/kg) B.W. of *Capparis spinosa L* extract for (4 weeks).
- **Group D**: alloxan-induced diabetes rabbits that were treated with (25 mg/kg) B.W. of *Capparis spinosa L* extract for (4 weeks).

Induction of Diabetes:

Diabetes was introduced experimentally in female laboratory rats by withholding food for (12 hours) approximately by a single subcutaneous injection of (125 mg / kg B.W) of alloxan monohydrate dissolved in distill water immediately before injection. The controlled animals received distill water only (Nimenibo-Vadia, 2003). Alloxan treated animals were allowed to drink of D-Glucose 5% overnight to prevent the potentially fatal hypoglycemia occurring as a result of massive insulin release following alloxan injection.
After seven days of injection the animals showing signs of extreme fatigue and frequent urination readied infected with diabetes (Alarcon-Aguilara et al., 2002).

**Blood Samples**

5mL of blood were drawn from each animal of experimental groups, by heart puncture method after 12 hours fast. Using 60 gauge syringes, the sample was transferred into clean tube, left at room temperature for 15 minutes for clotting, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in a clean tube in the refrigerator at 2-8ºC until the time of assay.

**Biochemical Measurement**

Enzymatic Colorimetric (GOD - POD) used to estimate the level of glucose in the blood serum. Sera were then collected and stored in freezer used in the determination of the levels of glucose. It is notable that all measurements were duplicated for each sample.

**Statistical Analysis:**

Statistical analysis was done using the software SPSS version 15.0; the results were expressed as mean ± standard deviations (mean ± SD). One way ANOVA-test was used to compare parameters in different studied groups. P values (P < 0.01) were considered statistically significant.

**Results and Discussion**

*Capparis spinosa* showed contain a number of antioxidant phytochemicals such as flavonoids and other polyphenols (Aslanturk and Tulay, 2009).

The chemical qualitative analysis tests results are shown in table (1) which indicate the presence of polyphenols, flavonoids and glycoside but the carbohydrate, tannins, saponins, alkaloids, terpinoids, terpenes and sterols gave a negative test, this ensure that polyphenolic compounds are pure. From table (1), it was found that glycosides because polyphenols is found normally connected with sacharids units. Figure (1) illustrates the UV-Vis spectra of the polyphenolic extract with max at (202nm) and another peak at (280nm), these peak absorbed at (202) for π-π* electronic transitions due to the founding of multi double bounds in aromatic rings for these compounds and another peak at (280) which is of low intensity appear in wave length longer than the first peak due to n- π* electronic transitions for non bonding electrons for oxygen atoms that are present in these compounds.
The plants extracts were analyzed to estimate their contents of polyphenol compounds. The identification of each compound was based on retention time in comparison with pure commercial standards (Shindalkar et al., 2005). The Figure (2) and fig (3) explained that the retention time of sample agrees with the retention time of the standard for most contents in the extract.
In this study and from HPLC results it was found that polyphenol extract contains some important compounds that include (gallic acid, caffeic acid, coumaric acid, Vanillic acid, Syringic acid and ferulic acid, Chlorogenic acid, Rutin and quercetin) as shown in fig (3) and the structures of these compounds were shown in table (2). The peaks of the mentioned chromatogram also pointed to presence of some unknown compounds that are thought represent derivatives of polyphenolic compounds.

Figure.2 HPLC chromatogram of standard polyphenolic compounds.
Figure.3 HPLC chromatogram of *Capparis spinosa* L phenolic extract.

Table.2 Concentration of standard polyphenolic compounds.

<table>
<thead>
<tr>
<th>seq</th>
<th>Polyphenolic contents in the standard</th>
<th>Retention time (min)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chlorogenic acid</td>
<td>1.09</td>
<td>40854</td>
</tr>
<tr>
<td>2</td>
<td>Caffeic acid</td>
<td>1.77</td>
<td>55315</td>
</tr>
<tr>
<td>3</td>
<td>Gallic acid</td>
<td>2.49</td>
<td>44057</td>
</tr>
<tr>
<td>4</td>
<td>Quercetin</td>
<td>3.18</td>
<td>39424</td>
</tr>
<tr>
<td>5</td>
<td>Syringic acid</td>
<td>4.24</td>
<td>45691</td>
</tr>
<tr>
<td>6</td>
<td>Coumaric acid</td>
<td>5.34</td>
<td>39763</td>
</tr>
<tr>
<td>7</td>
<td>Ferulic acid</td>
<td>6.09</td>
<td>37854</td>
</tr>
<tr>
<td>8</td>
<td>Vanillic acid</td>
<td>7.08</td>
<td>43141</td>
</tr>
<tr>
<td>9</td>
<td>Rutin</td>
<td>7.85</td>
<td>66993</td>
</tr>
</tbody>
</table>
### Table 3: Concentration of polyphenolic compounds in polyphenolic extract.

<table>
<thead>
<tr>
<th>seq</th>
<th>Polyphenolic contents in the extract</th>
<th>Retention time (min)</th>
<th>Area</th>
<th>Conc. of polyphenolic compounds µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chlorogenic acid</td>
<td>1.08</td>
<td>22004</td>
<td>40.395</td>
</tr>
<tr>
<td>2</td>
<td>Caffeic acid</td>
<td>1.75</td>
<td>54240</td>
<td>73.542</td>
</tr>
<tr>
<td>3</td>
<td>Gallic acid</td>
<td>2.49</td>
<td>32976</td>
<td>56.136</td>
</tr>
<tr>
<td>4</td>
<td>Quercetin</td>
<td>3.16</td>
<td>76898</td>
<td>146.289</td>
</tr>
<tr>
<td>5</td>
<td>Syringic acid</td>
<td>4.22</td>
<td>121635</td>
<td>199.659</td>
</tr>
<tr>
<td>6</td>
<td>P-coumaric acid</td>
<td>5.33</td>
<td>45022</td>
<td>84.918</td>
</tr>
<tr>
<td>7</td>
<td>Ferulic acid</td>
<td>6.08</td>
<td>65018</td>
<td>128.82</td>
</tr>
<tr>
<td>8</td>
<td>Vanillic acid</td>
<td>7.09</td>
<td>34199</td>
<td>59.454</td>
</tr>
<tr>
<td>9</td>
<td>Rutin</td>
<td>7.83</td>
<td>29482</td>
<td>33.003</td>
</tr>
</tbody>
</table>

### Table 4: Names and Structure of polyphenolic compounds in polyphenolic extract.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Structure</th>
<th>Organizational name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td><img src="image1.png" alt="Image" /></td>
<td>(1S,3R,4R,5R)-3-[(2Z)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4,5-trihydroxycyclohexanecarboxylic acid</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td><img src="image2.png" alt="Image" /></td>
<td>3-(3,4-Dihydroxyphenyl)-2-propenoic acid</td>
</tr>
<tr>
<td>Gallic acid</td>
<td><img src="image3.png" alt="Image" /></td>
<td>3,4,5-trihydroxybenzoic acid</td>
</tr>
<tr>
<td>Quercetin</td>
<td><img src="image4.png" alt="Image" /></td>
<td>2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one</td>
</tr>
<tr>
<td>Syringic acid</td>
<td><img src="image5.png" alt="Image" /></td>
<td>4-hydroxy-3,5-dimethoxybenzoic acid</td>
</tr>
<tr>
<td>P-Coumaric acid</td>
<td><img src="image6.png" alt="Image" /></td>
<td>3-(4-hydroxyphenyl)-2-propenoic acid</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td><img src="image7.png" alt="Image" /></td>
<td>3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td><img src="image8.png" alt="Image" /></td>
<td>4-Hydroxy-3-methoxybenzoic acid</td>
</tr>
</tbody>
</table>
Results of determination of serum glucose concentration in females rats groups (A, B, C and D) are given in table (5) and figure (4). A significant differences can be observed among (C and D) groups as compared with group (A) after treatment with (15 mg /kg B.W) and (25 mg /kg B.W) respectively of Capparis spinosa L extract for (4 weeks). Also there is a significant elevation that can be observed (P<0.01) in groups (B) as compared with group (A). The increase in blood glucose level is due to beta cell destruction by alloxan which lead to retardation of insulin production. Consequently, glucose can not enter into the cell leading to rise its level in the blood (Nelson and Cox, 2000 ; Decarvalho et al., 2003). The levels of serum glucose decreased significantly in (C and D) groups as compared to group (B) after treatment (4 weeks) days with (15 mg /kg B.W) and (25 mg /kg B.W) respectively. The antidiabetic activity of Capparis spinosa L. leaves may be due to the presence of polyphenoilc compounds that have hypoglycemic activity in the body (Lemhadri et al., 2007). Treatment with antioxidants may prevent or ameliorate abnormal function and biochemistry of nerve and protect nerves against to free radicals damage (Karasu et al., 1995). Consequently it is postulated that these agents can prevent diabetic complications (Montonen et al., 2004). Phenolic acids are well-known antioxidants, and also their antidiabetic activities have been reported in many studies( Mukherjee et al., 2006; Aslan et al., 2007). As that herbal extracts containing flavonoids was reported to demonstrate antidiabetic activity (Suba et al., 2004). On the basis of the above evidence, it is possible that the flavonoids present in this plant may be responsible for the observed antidiabetic activity.

Table.5 Effects of polyphenolic extract on serum glucose levels after treatment with polyphenolic extract (15mg/kg B.W) and (25 mg/kg B.W).

<table>
<thead>
<tr>
<th>Glucose cone mg/dl</th>
</tr>
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<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>L.S.D</td>
</tr>
</tbody>
</table>
Conclusions

At the end of this thesis points below can be concluded:

- Polyphenolic extract isolated from *Capparis spinosa* L. leaves, in this study giving the immediately yields.
- Compounds that are exist in each extract identified by using UV-Visible spectrum, also appearance of several peaks indicating to the existing of phenolic acids.
- Compounds are determined through using HPLC technique, where in polyphenolic extract seven phenolic acids one flavonoid and one glycosides (Gallic acid, Caffeic acid, Coumaric acid, Vanillic acid, Syringic acid and Ferulic acid, Chlorogenic acid, Rutin and Quercetin) have been extracted from *Capparis spinosa* L. leaves.
- The extracted compounds were found to reduce serum glucose in alloxan induced diabetes mellituse females rats.

References


